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Reproductive effort reduces specific immune response and parasite resistance

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If a trade-off exists between reproductive effort and immune function, life-history decisions may have important implications for parasite resistance. Here, we report effects of experimental manipulation of reproductive effort on subsequent specific immune function and parasite resistance in the collared flycatcher, *Ficedula albicollis*. Our results show that increased reproductive effort of females immunized with Newcastle disease virus (NDV) vaccine negatively affected the ability to respond with NDV-specific antibodies. We further show that increased reproductive effort increased the intensity of *Haemoproteus* infections and that such infections are associated with higher mortality. Our results thus provide support for the hypothesis that immune suppression caused by reproductive effort may be an important mechanism mediating the life-history cost of reproduction.

Keywords: *Ficedula albicollis*; cost of reproduction; parental effort; specific immune response; Newcastle disease virus; *Haemoproteus*

1. INTRODUCTION

Life-history theory assumes that components of reproductive effort, such as production of offspring and parental care, are costly and are achieved at the expense of resources otherwise available for future reproduction (Williams 1966). For birds and mammals, it has been shown experimentally that high parental investment in reproduction can affect survival of the parents (Nur 1984; Dijkstra *et al*. 1990; Daan *et al*. 1996; Nordling & Gustafsson 1998) as well as their future fecundity (Clutton-Brock *et al*. 1983; Gustafsson & Sutherland 1988; Gustafsson & Pärt 1990). Thus, to maximize lifetime reproductive success, organisms face the basic question of what proportion of available resources should be invested in reproduction now as compared to saved for a later occasion. The best strategy for an individual organism depends on many factors, such as environmental variables, genetic constraints and social interactions, and may range from investing nothing (forgoing breeding) to investing all, so as to cause death after reproduction (Stearns 1992). It is obvious that organisms incur a cost of reproduction that interacts with their life histories (Roff 1992), but knowledge of the physiological basis of this cost is very limited.

Recently, efforts have been made to experimentally study physiological mechanisms potentially involved in mediating the costs of both life-history and secondary sexual characters in natural populations. Research is currently focused on energy/nutritional trade-offs (Deerenberg *et al*. 1995; Nilsson & Svensson 1996; Verhulst & Tinbergen 1997) and immunological mechanisms mediating the costs of reproduction (Gustafsson *et al*. 1994; König & Schmid-Hempel 1995; Saino & Møller 1996; Deerenberg *et al*. 1997; Nordling & Gustafsson 1998). In this study, we concentrate on the latter mechanism, the reproductive trade-off with immune function.

Among the various physiological factors that are likely to be involved in mediating the cost of reproduction, immune suppression stands out as a possibly important mechanism as it offers several pathways by which reproductive effort may be linked to long-term negative effects on viability and reproduction. Such long-term effects of reproductive effort are difficult to explain physiologically, in terms of energy or nutritional depletion alone, because the effect of malnutrition on bodily functions is, in most cases, a reversible process. However, it has long been known that stressful conditions, e.g. strenuous physical exercise, malnutrition and social stress, may lead to a suppression of immune functions, such as response of lymphocytes to mitogens *in vitro*, or the ability to recover from contracted infections (Gross & Siegel 1973; Chandra & Newberne 1977; Gershwin *et al*. 1985; Cooke 1993). High reproductive effort may indeed be considered as a stressful condition in several aspects and this, in turn, may impose not only immediate effects but possibly also life-long negative consequences for viability and reproduction. In principle, there are several ways in which these long-term effects may develop. For example, immunosuppression may reduce acquired immunity for contracted infectious agents, increase the risk of a chronic course of infections or decrease the defence against pathogenic effects causing lesions to organs with limited recovery functions. Immunosuppression may potentially also cause increased risk of malignancy and autoimmune disease, for example by contraction of infectious agents which are oncogenic or

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structurally similar to auto-antigens (Jenkins *et al*. 1988; Oldstone 1989; Beverlay 1993; McNagny & Graf 1996).

A number of studies have suggested that increased reproductive effort may be associated with increased parasite load (Festa-Bianchet 1989; Møller 1993; Norris *et al*. 1994; Richner *et al*. 1995; Oppliger *et al*. 1996). Since immunosuppression is known to causally increase host susceptibility to infectious diseases (Rosen 1993), a trade-off between reproductive effort and immune function could be an important mechanism explaining the reduced parasite resistance observed in these studies. An alternative explanation may be altered behaviour where additional foraging demands leads to increased exposure to parasites, e.g. by increased social and environmental interactions or reduced preening activity (Clayton 1991).

For the present study we have used a population of collared flycatchers, *Ficedula albicollis*, breeding in the southern part of the Baltic island of Gotland, Sweden. Earlier studies of reproductive trade-offs in the collared flycatcher (Nordling & Gustafsson 1998) and the American kestrel, *Falco sparverius* (Apanius 1991), have shown that increased reproductive effort reduces immunoglobulin synthesis and leukocyte proliferation. These data provided evidence for a trade-off between reproductive effort and resources allocated to immune function in general. Here, we aim to test whether the reproductive effort of collared flycatchers negatively affects the ability to mount a specific immune response against a potential pathogen. The existence of such a mechanism has been demonstrated by laboratory experiments on captive zebra finches, *Taeniopygia guttata*, with a negative relationship being found between immune responsiveness and parental effort as caused by manipulated brood sizes (Deerenberg *et al*. 1997).

The idea that increased infectious disease, via immune suppression mechanisms, is an important component mediating the cost of reproduction necessitates that reproductive effort can be shown to affect both immune function and parasite resistance. This study includes experimental testing of those two predictions. We present results showing effects of experimentally changed reproductive effort on the specific immune function. Results from an equivalent effort experiment are also presented showing effects on the resistance to a naturally occurring parasite associated with negative fitness consequences. First, we show that our choice of immunogen, the virus causing Newcastle disease (paramyxovirus serogroup 1), can provoke an immune response in the collared flycatcher. A monoclonal-antibody-blocking enzyme-linked immunosorbent assay (ELISA) was used for the detection of NDV-specific antibodies. Second, by experimental manipulation of reproductive effort of NDV-immunized females, we test the hypothesis that an increase in reproductive effort will lead to a decrease in the bird's ability to mount a specific immune response to the antigen. Third, we analyse effects of experimental manipulation of reproductive effort on prevalence and intensity of *Haemoproteus*, a blood parasite that often causes infections in birds. Finally, we look for pathogenic effects of such infections in an age-controlled study of mortality and prevalence of infection in non-manipulated female collared flycatchers.

2. MATERIAL AND METHODS

(a) *Study area and species*

This study was carried out using a population of collared flycatchers, *Ficedula albicollis*, breeding in the southern part of the Baltic island of Gotland, SE Sweden (57°10′ N, 18°20′ E). The collared flycatcher is a small (*ca*. 13 g), migratory, insectivorous passerine bird, which breeds throughout most of eastern and central Europe, with isolated populations on the Baltic islands of Gotland and Öland. Females lay a clutch of 4–8 eggs. Only a single brood is reared by females annually. For several reasons, few species are more amenable to field experimentation. Nest boxes are strongly preferred over natural cavities, making it possible to attract almost the whole breeding population to boxes. The collared flycatcher is easy to catch and only rarely abandons a breeding attempt after having been caught and blood sampled. In the isolated population on Gotland, both adults and young show remarkably high site fidelity. As a result, survival can be assessed with exceptional accuracy (Gustafsson 1989). For further details on area and species, see Pärt & Gustafsson (1989).

(b) *Manipulation of reproductive effort*

We altered the reproductive effort of female collared flycatchers by manipulating the number of offspring in their broods. Treatments were randomly assigned to individuals with the same hatching date and clutch size. This experimental technique is identical to that which has been used successfully in the past to demonstrate reproductive trade-offs (e.g. Gustafsson & Sutherland 1988). On the second day after hatching, two nestlings were transferred from their natal nest to a foster nest. In controls, two nestlings were exchanged between nests so that brood size did not change. As a result, three matched groups of females were formed with reduced, unchanged or increased brood sizes within the limits of natural variation. To verify that experimental females experienced different rates of reproductive effort as a consequence of manipulated brood sizes, the number of fledged young was analysed in relation to experimental groups.

(c) *Immunization method and sample preparation*

To test the specific immune function we immunized females with a vaccine against Newcastle disease (Nobi®-Vac-Paramyxo) and measured their ability to respond with NDV-specific antibodies. The NDV vaccine is an oil emulsion with formalin-inactivated paramyxovirus serotype 1 (PMV-1). Avian PMV-1 is the most important pathogen in poultry (Alexander 1997). The virus has been demonstrated in at least 236 species from 27 of the 50 orders of birds. The most common carriers include free-ranging waterfowl, Pittidae, Psittaciformes, some Passeriformes and Strigiformes (Gerlach 1994).

Females were immunized three days before expected hatching of their clutch. For injection of the NDV vaccine, we used a 25 µl Hamilton® syringe with Luer-lock and sterile, single-use Microlance® 3 needles (0.30 mm × 13 mm). Before injecting the vaccine the bird's tarsus was gently fixated. Females were then given a subcutaneous injection of 7 µl (7 mm^2) NDV vaccine on the inside of the right thigh. To avoid regurgitation of antigen, a skin lock was created by *ca*. 1 cm of subcutaneous tunnelation before injecting the NDV vaccine. The subcutaneous injection technique, in comparison with intraperitoneal or intramuscular injections, offers not only accurate and safe application of vaccine in the tissues, but avoids unnecessary suffering of the experimental animals as well.

Two weeks later, on the 12th day of rearing the young, females were blood sampled for evaluation of immune responses. To ensure a long-term effect of vaccination, the females were also given a second 7 µl vaccine injection (after blood sampling) in case they could be recaptured and tested for immune responses the following year. Blood samples were obtained by extracting 75–100 µl blood from the cutaneous ulnar vein and collected into plastic tubes (Microvette®) powdered with potassium salt of EDTA to avoid unnecessary anticoagulant dilution of the blood. The cell elements of the blood were separated from plasma by careful centrifugation of the plastic tubes to avoid haemolysis. The plasma was transferred to kryo-tubes and stored between −70 °C and −20 °C until processed with immunoassay.

(d) *Specific immune responses*

To determine the amount of NDV-specific antibodies, we used a novel type ELISA, a monoclonal-antibody-blocking ELISA (Czifra *et al*. 1996). The test has been developed and evaluated for diagnosing the infection in poultry, but it can be used to test sera from other birds as well (Koch *et al*. 1998). The commercially available test (SVANOVA Biotech, Uppsala Science Park, Uppsala, Sweden) has been modified to meet the special requirements of this study. In short, sample volume, conjugated monoclonal antibody and the substrate solution were reduced to 25 µl and new cut-off values were established to achieve the best specificity and sensitivity by testing 41 sera from non-vaccinated, non-infected collared flycatchers. We found no evidence of naturally occurring Newcastle disease among analysed birds and PMV-1 may therefore be considered a novel antigen for the collared flycatcher. Optical density (OD) values of test sera were compared with the OD value of an NDV-negative serum and the proportions were described as percentage inhibition (PI) values. By definition, PI can be anything between 0 and 100. Higher PI means more NDV-specific blocking antibodies in the actual sample.

(e) *Evaluation of immune responses to the NDV vaccine*

Experimental brood manipulation of NDV-immunized females was performed in 1995. This was the first year we immunized females with NDV vaccine and consequently no female had received NDV vaccine before. However, to be able to present more detailed information about the validity of these results, we repeated the immunization procedures in the experimental area the subsequent year. Females raising their natural brood sizes were immunized according to an identical protocol as the year before. All females were immunized three days before hatching of their clutch. We analysed blood samples that were taken both at this stage as well as two weeks later to be able to test if NDV antibodies increased as a result of immunization (i.e. a 'before and after' treatment design). Because some of the females were found to have been vaccinated in the preceding year, they were tested in groups according to the number of injections received the previous year. Of a total of 54 females, 38 had not been vaccinated previously and consequently they were immunized for the first time in 1996 (and thus were comparable with NDV-vaccinated females rearing experimental broods in 1995). Four females had received one injection the previous year and 12 had received two injections.

(f) *Parasite prevalence and intensity*

We chose to screen for *Haemoproteus* as an indicator of infection status. Species of *Haemoproteus* are the most common haemosporidians encountered in wild birds (Atkinson & Riper 1991). In the collared flycatcher population the estimated prevalence in adult birds is 20%. Haemoproteids have a life cycle similar to that of *Plasmodium*, but they differ in two basic aspects: (i) *Haemoproteus* species are transmitted by *Culicoides*, and (ii) there is an absence of merogonic stages in the circulating blood (Desser & Bennet 1993). In the present study, we screened for *Haemoproteus* infections by microscopic examination of parasite-concentrated blood films (Bennett 1962). This method has a higher diagnostic sensitivity than the standard blood smear technique, but it cannot be used for intensity measurements (Bennett 1962). Samples (20 µl) of blood in heparin-coated microcapillary tubes (Microcaps®) were transported in cooling boxes to the field laboratory and centrifuged for 12 min at a speed of 10 000 r.p.m. in a standard micro hematocrit centrifuge. By this separation procedure, erythrocytes containing *Haemoproteus* parasites accumulate just underneath the buffy coat. This fraction of the blood column was cut out and smeared on a slide, air-dried, methanol fixated and Giemsa stained. One hundred microscopic fields were scanned (× 100 oil-immersion).

To measure the concentration of *Haemoproteus* in peripheral blood, we used microscopic examination of standard blood smears. In the collared flycatcher, *Haemoproteus* is the only haematozoan that can be sufficiently detectable in this way and thus allow measurement of parasite intensity. Approximately 5 µl of blood, obtained from the cutaneous ulnar vein, was immediately applied on a glass slide and smeared out with a faceted glass tool by standard wedge technique. These smears were made directly in the field and within a few minutes after obtaining blood samples from the birds. In a homogeneous erythrocytic monolayer, the number of infected red blood cells per 100 microscopic fields were counted (Giemsa stain, × 100 oil-immersion). This measure of *Haemoproteus* intensity has shown a very high repeatability (Allander & Bennett 1994). Slides were analysed by the late G. F. Bennett at the International Reference Centre for Avian Haematozoa, Newfoundland, Canada.

(g) *Mortality*

Female collared flycatchers that were not subjected to experimental manipulation were blood sampled while feeding young and screened for prevalence of *Haemoproteus* infections ($n = 272$). We compared survival rates of groups of females classified as either infected or not infected by *Haemoproteus*. Females not returning to the breeding area for two successive years were classified as dead. This assumption is justified, based on an analysis showing that females not found after two years have a probability of only 0.4% ($n = 260$) of being found breeding later. In view of the high fidelity to their breeding grounds, the likelihood that the missing females would breed somewhere else is negligible (Pärt & Gustafsson 1989). Samples from two successive breeding seasons were pooled. To avoid pseudoreplication, we excluded 15 females from a random year. Thus, all birds were only entered once in the pooled sample for statistical analysis. To control for age-related effects on mortality, we analysed one-year-old females and older females separately.

3. RESULTS

(a) *Test of immunization method and immunoassay*

Females immunized with 7 µl NDV vaccine in 1996 and no previous history of vaccination showed a significant increase in levels of NDV-specific antibodies two

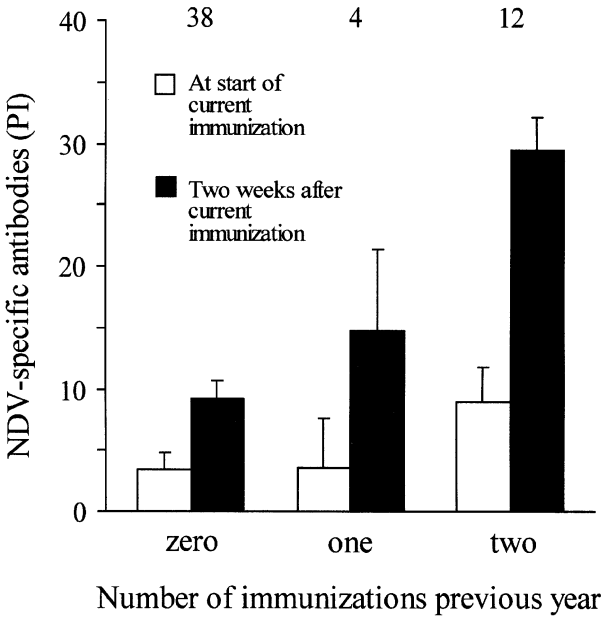

Figure 1. The level of NDV-specific antibodies of female collared flycatchers before and after immunization with NDV vaccine in 1996 (means±s.e.). Females were blood sampled (for initial values of NDV-specific antibodies) and immunized three days before hatching of their clutch. At the 12th day of rearing the young, females were blood sampled once again for response values of NDV-specific antibodies. Females are divided into three groups (zero, one and two) according to the number of vaccine injections received the previous year, 1995. Sample sizes are given above bars. Optical density (OD) values of each test sera were compared with the OD of an NDV-negative serum and the proportions were described as percentage inhibition (PI) values. Higher PI means more NDV-specific blocking antibodies in the actual sample.

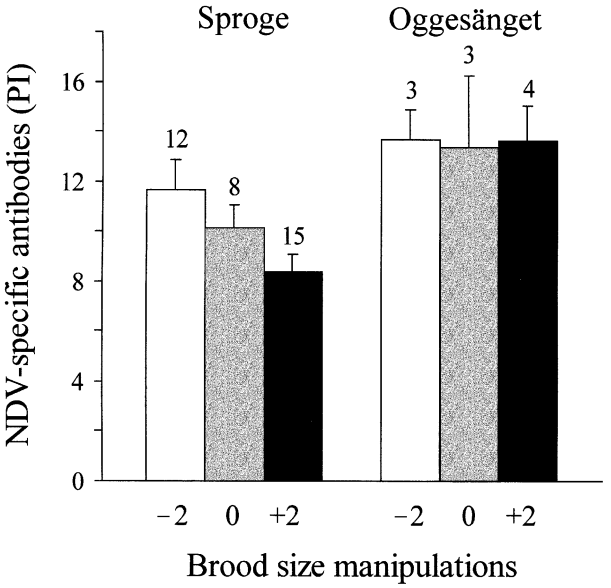

Figure 2. Levels of NDV-specific antibodies of female collared flycatchers after immunization with NDV vaccine in 1995, in relation to experimental brood sizes in the same year (means±s.e.). Females were immunized three days before hatching of their clutch. At the 12th day of rearing reduced (−2), control (0) and increased (+2) brood sizes, females were blood sampled for response values of NDV-specific antibodies. Data are presented from two different habitats, Sproge and Oggesänget. Sample sizes are given above bars.

weeks later (paired t-test: d.f.=37, $p=0.0058$; see figure 1). In addition, females immunized in the previous year, 1995, showed an increase in NDV-specific antibodies as a response to vaccination in 1996 (paired t-test: d.f.=15, $p=0.0036$; see figure 1). There was also a positive relationship between the increase in NDV-specific antibodies and the number of immunizations the previous year (ANOVA: $F_{2,51}=4.16$, ordered heterogeneity (o.h.) test: $r_sP_c=0.979$, $p=0.0035$; see figure 1). We used an ordered heterogeneity test (Rice & Gaines 1994) because the mean response is expected to increase in relation to the number of previous immunizations (Glick 1986). Further, the initial levels of NDV-specific antibodies tended to be higher in females given two injections the previous year (ANOVA: $F_{2,51}=1.98$, $p=0.14$).

(b) *Relationship between reproductive effort and primary specific immune response*

Experimental manipulation of the reproductive effort of NDV-immunized females was carried out in two different breeding habitats, Sproge and Oggesänget, separated by a distance of approximately 30 km. At the end of the nestling feeding period, the mean number of fledged young in the nests were still quantitatively different between experimental groups indicating that experimental females indeed experienced different rates of reproductive effort. The mean number (±s.e.) of fledged young per nest in reduced broods was 2.7±0.34, in controls it was 4.1±0.48 and in increased broods it was 5.1±0.56 (ANOVA: $F_{2,42}=6.04$, $p=0.0049$). The level of NDV-specific antibodies showed a normal distribution (Shapiro–Wilks: $W=0.97$, $n=45$, $p>0.05$) and homoscedasticity (Bartlett's test: $\chi^2=4.49$, d.f.=2, $p>0.05$). We found a significant effect of habitat and experimental brood size on the NDV-specific antibody response (ANCOVA: area, $F_{1,42}=10.0$, $p=0.0029$; experimental brood size, $F_{1,42}=5.5$, $p=0.023$). In Sproge, there was a clear negative relationship between experimental brood size and the level of NDV-specific antibodies (ANOVA: $F_{2,32}=3.42$, o.h. test: $r_sP_c=0.955$, $p=0.0075$; see figure 2). In Oggesänget, no relationship was found (ANOVA: $F_{2,7}=0.01$, o.h. test: $r_sP_c=0.004$, $p=0.49$; see figure 2) possibly due to small sample size ($n=10$). We used ordered heterogeneity tests (Rice & Gaines 1994) because the expected rank order of means was specified by the hypothesis tested of an inverse relationship between the ability to mount a specific immune response and experimental brood sizes.

(c) *Reproductive effort and infection*

To test if increased reproductive effort is associated with greater susceptibility to infections, as suggested by its observed negative effects on immune function, we studied the effects of brood manipulations on infection status in non-immunized female collared flycatchers (the sample size of immunized birds was too small to detect parasite effects; the prevalence of *Haemoproteus* in the collared flycatcher population is estimated to be 20%). However, using non-immunized females in this analysis has the advantage that conclusions drawn from the effects

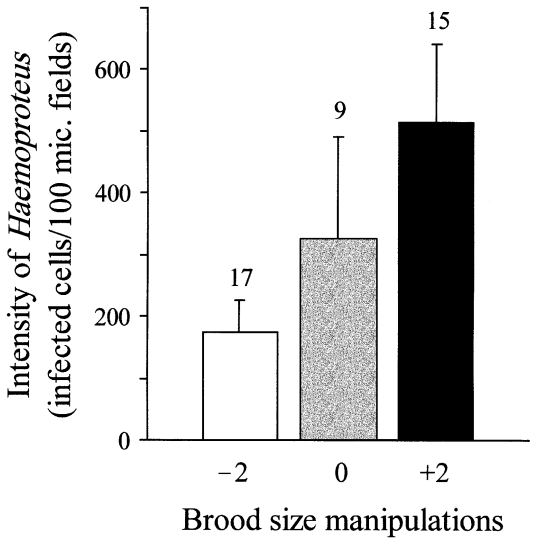

Figure 3. Concentration of *Haemoproteus* parasites in peripheral blood of female collared flycatchers rearing reduced (−2), control (0) and increased (+2) brood sizes (means ± s.e.). Blood samples were taken at the end of the nestling feeding period. Sample sizes are given above bars.

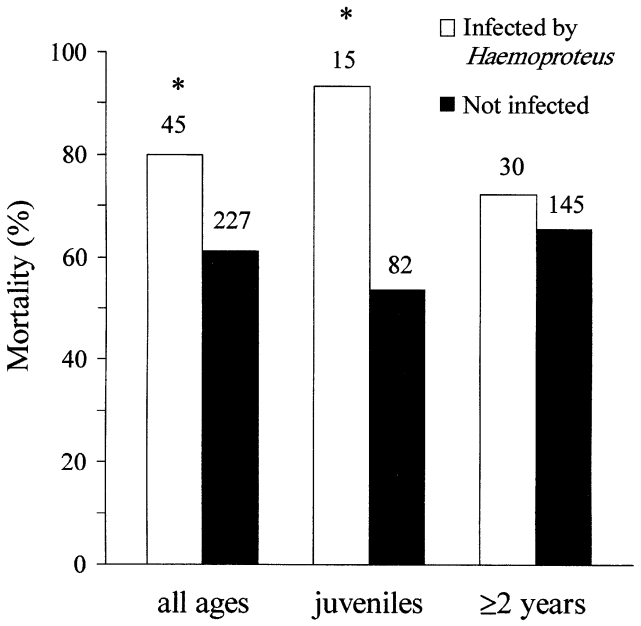

Figure 4. Difference in annual mortality rates between females infected by *Haemoproteus* and uninfected controls. The first pair of bars represent all females in the test sample (all ages), the second pair one-year-old females (juveniles), and the third pair older birds (⩾2 years). Asterisks indicate significant differences in mortality rates (χ^2_c, $p<0.05$, Cochran's rule not violated). Sample sizes are given above bars.

of brood size manipulation on parasite resistance are not confounded by possible effects of the vaccination on parasite resistance in general.

Non-immunized females subjected to a brood size experiment were blood sampled and screened for naturally occurring *Haemoproteus* infections at the end of the nestling feeding period. The mean number (±s.e.) of fledged young per nest in reduced broods was 3.6±0.14, in controls 5.3±0.24, and in increased broods 7.3±0.22 (ANOVA: $F_{2,152}=95.7$, $p<0.0001$). We found no effects on prevalence of *Haemoproteus* infections caused by experimental treatment ($\chi^2=1.3$, d.f.=2, $n=155$, $p=0.52$). However, females infected with *Haemoproteus* showed a positive relationship between experimental increase in reproductive effort and intensity of infection (Kruskal–Wallis: $H=6.05$, d.f.=2, o.h. test: $r_sP_c=0.951$, $p=0.008$; see figure 3). (We used a non-parametric test as log transformation of *Haemoproteus* intensity could not compensate for skewness when tested by Shapiro–Wilks test: $W=0.93$, $n=41$, $p<0.05$.) For these females, the mean number (±s.e.) of fledged young per nest in reduced broods was 3.4±0.26, in controls 4.3±0.78, and in increased broods 6.5±0.45 (ANOVA: $F_{2,38}=14.3$, $p<0.0001$).

(d) *Infection and mortality*

The observed relationship between reproductive effort and intensity of *Haemoproteus* infection suggests a possible causal role of infectious agents in mediating a cost of reproduction in the collared flycatcher. This suggestion assumes that *Haemoproteus*, or some associated infectious agent, has negative viability effects on its host. We therefore tested if naturally occurring *Haemoproteus* infections were associated with elevated mortality. We found that females suffering from *Haemoproteus* infections indeed showed increased mortality (all ages: $\chi^2_c=4.98$, d.f.=1, $n=272$, $p=0.026$; see figure 4). This difference in mortality was even more pronounced in the separate analysis of one-year-old females (juveniles: $\chi^2_c=6.73$, d.f.=1, $n=97$, $p=0.0095$; see figure 4). Older females infected with *Haemoproteus* showed only a non-significant increase in mortality (⩾2 years: $\chi^2_c=0.38$, d.f.=1, $n=175$, $p=0.54$; see figure 4).

4. DISCUSSION

To show more specifically that vaccination actually leads to increased levels of NDV-specific antibodies, 54 females were vaccinated the year after the experiment. Of those females, 38 received their first injection while 16 had been vaccinated the previous year (four had been vaccinated once and 12 twice). Females receiving their first NDV injection (the 'zero' group in figure 1) responded with a significant increase of NDV-specific antibodies. One vaccination in the previous year did not seem to influence the basic level of NDV antibodies before vaccination, but the response to immunization was increased at the second year (the 'one' group in figure 1). The 12 birds that were vaccinated twice the previous year tended to have higher basic values and the increase of the antibody level was even stronger (the 'two' group in figure 1). This is in good agreement with the common knowledge about the differences between a primary and secondary immune response. But more importantly, we show that the level of NDV-specific antibodies significantly increased in response to vaccination.

All females in the brood size experiment were immunized with NDV vaccine for the first time. We found an inverse relationship between experimental brood size and the level of NDV-specific antibodies at the end of the nestling feeding period. These experimental data show that increased reproductive effort of collared

flycatchers, within the limits of naturally occurring variation, will reduce the specific immune response to a potential pathogen. Laboratory experiments on captive zebra finches, challenged with intraperitoneal injections of sheep red blood cells, have recently shown similar effects on specific immune responses as a consequence of experimentally increased brood sizes (Deerenberg *et al*. 1997). To our knowledge, however, our data provide the first experimental demonstration of reproductive suppression of specific immune responses in a natural population.

In the Sproge population, females showed an inverse relationship between reproductive effort and specific immune response to NDV vaccine. In Oggesänget, by contrast, we found no such relationship (note the small sample size). Notably, there was a significantly higher mean level of immune response among the females from Oggesänget than among those from Sproge. Because good nutritional status is important for the ability to mount an effective immune response (Chandra & Newberne 1977; Gershwin *et al*. 1985; Lochmiller *et al*. 1993), this difference suggests that Oggesänget, at least in this particular year, may have offered superior food abundance in relation to Sproge. Assuming that this suggestion is correct, the increase of reproductive effort in terms of enlarged brood sizes in Oggesänget may not have been sufficient to explore a trade-off with immune function. Although we have no data to support this suggestion, we believe that environmental factors may influence the extent to which reproductive suppression of immunity operates in natural populations.

We found no relationship between manipulated brood sizes and prevalence of *Haemoproteus* in the collared flycatcher females. The time period between brood manipulation and blood sampling was 9 ± 2 days. Considering the long prepatent period for *Haemoproteus* infections (between two and three weeks (Desser & Bennett 1993)), effects of brood manipulation on the prevalence of *Haemoproteus* in the bird population should not be expected. However, reduced ability of the immune system to control existing infections, by suppressing parasite replication, can be supposed to be a rapid effect of immune suppression, and thus possible to detect within the short time span of our experiment. Indeed, this is what we found. Females infected with *Haemoproteus* showed a positive relationship between experimental increase in reproductive effort and intensity of infection. On average, females rearing enlarged broods had a three-fold increase in *Haemoproteus* intensity in relation to females rearing reduced broods. A similar increase in parasite intensity as a consequence of experimentally manipulated parental effort has been recorded in studies of barn swallows (Møller 1993) and great tits (Allander 1997) as well. This suggests the general operation of a reproductive trade-off with parasite resistance, presumably via immune suppression. In studies where brood size manipulations have been found to affect the prevalence of blood parasites (Norris *et al*. 1994; Richner *et al*. 1995; Oppliger 1996; Allander 1997), it is possible that low-level parasitaemia were not detected in the blood smears (Atkinson & Riper 1991). If this suggestion is correct, the increased prevalence found in these studies could reflect an increased parasite intensity among already infected birds.

The increased mortality of young females suffering from *Haemoproteus* infections show that *Haemoproteus* may possibly play a role in mediating negative effects on viability and reproduction in the collared flycatcher. However, it is not likely that reduced parasite resistance should be confined to *Haemoproteus* alone, and we have no experimental evidence of a causal link between *Haemoproteus* and mortality. Although it has been shown experimentally that *Haemoproteus* can have severe pathogenic effects in its avian host (Atkinson *et al*. 1988), haemoproteids are most commonly associated with low pathogenicity (Atkinson & Riper 1991; Desser & Bennett 1993). It is reasonable to assume though, that birds infected with one pathogen, e.g. *Haemoproteus*, have increased likelihood of being infected by yet another, as poor immune function may be considered as a common cause of infection for a wide array of potential pathogens. Thus, *Haemoproteus* infections may be viewed as an indicator of low parasite resistance in general and the increase in intensity of infection as a quantitative measure of such resistance. The mortality effects associated with *Haemoproteus* infections were evident only in young females. Older females with *Haemoproteus* infections showed a moderate (non-significant) increase in mortality. This suggests not only a process of selection sorting out individuals incapable of immunological control of initial infections, but also the possibility that parasite-mediated costs of reproduction decrease with age. Suppose that older birds, as a consequence of selection and acquired immunity, have superior defence control of the infectious agents in their natural environment in comparison to younger birds. If this is the case, it is likely that older birds can afford a relatively higher reproductive-effort-induced immune suppression and still be able to sustain acceptable resistance to parasite infections.

Our experimental data test for causality between reproductive effort and specific immune function as well as between reproductive effort and parasite resistance. We do not test for causality of immune suppression in *Haemoproteus* resistance, which would require manipulation of immune function itself (Norris *et al*. 1994; Sheldon & Verhulst 1996). However, numerous studies have shown that immunosuppression causally increases host susceptibility to infectious diseases in general (Rosen 1993). Recent reviews have also underlined that infectious agents in general can have severe negative effects on a wide range of fitness components of their avian hosts (Møller *et al*. 1990; Loye & Zuk 1991). Therefore, the observed negative effects of reproductive effort on specific immune function, as well as parasite resistance, can potentially be explained as follows. Immune suppression caused by increased reproductive effort, within the limits of natural variation, can be sufficiently strong to provoke a significant increase in the infection intensity of parasites with substantial pathogenic effects in the hosts. This chain of reactions may prove to be an important pathway for the life-history cost of reproduction.

The traditional explanation of negative fitness consequences of parasitism has focused on the pathogenicity of infectious agents. However, if trade-offs exist between life-history traits, immune function and parasite resistance, a more complicated picture emerges. Adaptive changes in reproductive effort of infected hosts may

account for both inverse and positive relationships between host reproductive output and prevalence of infection (Forbes 1993). First, the pay-off for a reduction in reproductive effort may be increased parasite resistance with positive effects on current as well as future reproductive success. The contracted infection will be kept under control and the parents will be able to raise at least a few offspring and/or the prospects for survival may increase affecting future reproductive success positively. Second, the potential pay-off for an increase in reproductive effort when infections are contracted may depend on many factors, such as the prospects for future reproduction, exposure and type of infectious agent and the physiological costs and trade-offs associated with fighting a particular infection. Such a decision may potentially incur smaller fitness costs than cutting down on resources allocated to reproduction and thereby reducing the reproductive output. Tests of these adaptive host responses have implications for studies of both parasite–host interactions and the evolution of adaptive phenotypic plasticity, and they are a challenge for future experimental investigations. However, the basic assumption underlying these theories of host–parasite interactions is that reproductive effort and immune function trade off against each other with consequences for parasite resistance and host fitness. We believe that the results presented in this study, where experimental manipulation of reproductive effort affected both specific immune function and parasite resistance, provide evidence supporting this assumption.

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